

cartilage degradation and confirms a link between a native biological pathway and pharmacology which translates to human tissues and non-human primate models, subsequently pointing to a specific OA patient population to target. However, our results suggest selective and potent ADAMTS-5 engagement/inhibition with GSK2394002, while demonstrating desired effects on cartilage-related endpoints, results in modulation of CV functions that pose considerable challenges for clinical development.

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COMPARISON OF THE CELLULAR AND CYTOKINE CONCENTRATIONS IN THE OUTPUT OF THE AUTOLOGOUS PROTEIN SOLUTION, ORTHOKINE, AND ONOCOMMED 2 DEVICE SYSTEMS

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Purpose: The purpose of this study was to characterize the cellular and cytokine concentrations in the outputs of the Autologous Protein Solution (APS), Orthokine, and Onocommed 2 Device Systems. Anti-inflammatory cytokines and anabolic growth factors were analyzed due to their potential role in inhibiting damaging inflammation in osteoarthritis (OA) and inducing disease-modifying cartilage repair.

Methods: Blood from 6 human donors (WIRB Study # 1115097) was drawn and allocated so that blood from each donor was used for 1) whole blood analysis, 2) processing with the APS Device System (Biomet, Warsaw, IN, USA), 3) Orthokine Device System (Orthogen Lab Services, Dusseldorf, Germany), and the Onocommed 2 Device System (Plasmaconcept AG, Bonn, Germany). Each device system was processed according to the manufacturer's instructions. Cellular content was analyzed using a Cell Dyn 3700 hematology analyzer (Abbott Laboratories, Chicago, IL, USA). Cytokine content was measured using enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN, USA).

Results: The APS Device System produced a solution with greater concentrations of white blood cells (WBC) and platelets (PLT) than whole blood, the output of the Orthokine Device System, and the Onocommed 2 Device System ($p < 0.05$, t-test). The output of the APS Device System contained significantly decreased red blood cell (RBC) concentrations than whole blood, but greater than the RBC concentrations in the conditioned serums produced by the Orthokine and Onocommed 2 Device Systems ($p < 0.05$, t-test) (Table 1).

The APS Device System produced solutions with significantly greater concentrations of anti-inflammatory cytokines than the Orthokine and Onocommed 2 device systems (Figure 1). For example, the concentration of interleukin-1 receptor antagonist (IL-1ra) in the output of the APS device system was $54,922.1 \pm 32,750.7$ (pg/ml), whereas the output of the Orthokine device system was $1,618.0 \pm 674.7$ (pg/ml) and the output of the Onocommed 2 device system was 277.1 ± 338.2 (pg/ml). The concentration of IL-1ra in the outputs of the Orthokine and Onocommed 2 device systems were significantly less than the IL-1ra concentration in whole blood ($5664.5 \pm 2,317.6$ pg/ml) ($p < 0.05$). The output of the APS device system also contained significantly greater concentrations of soluble interleukin-1 receptor antagonist type II (sIL-1RII), soluble tumor necrosis factor type I (sTNF-RI), and sTNF-RII ($p < 0.05$, t-test) (Figure 1).

The APS Device System produced solutions with similar or significantly greater concentrations of anabolic growth factors compared to the other device systems. For example, the APS Device System produced a solution that contained $13,575.9 \pm 5,959.2$ (pg/ml) platelet-derived growth factor-BB (PDGF-BB). The Onocommed 2 and Orthokine device systems had outputs containing $2,302.3 \pm 1,022.6$ and $2,765.5 \pm 1,101.2$ (pg/ml) PDGF-BB, respectively ($p < 0.05$, t-test). The APS Device System also produced solutions with significantly greater concentrations of PDGF-AB, transforming growth factor- β (TGF- β), and epidermal growth factor (EGF) compared to the Onocommed 2 Device System ($p < 0.05$, t-

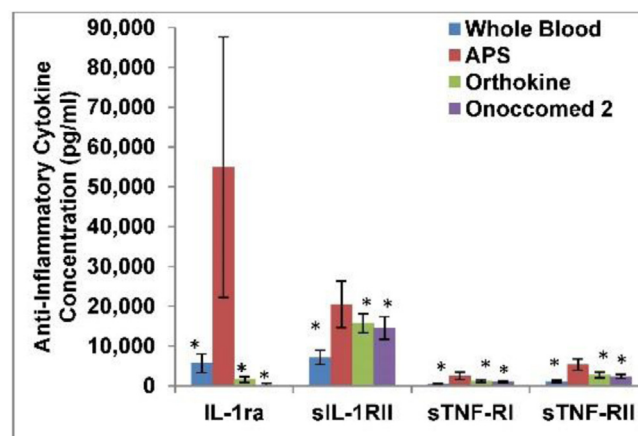


Figure 1. Anti-inflammatory cytokine concentrations.* significantly < APS Device System ($p < 0.05$, t-test).

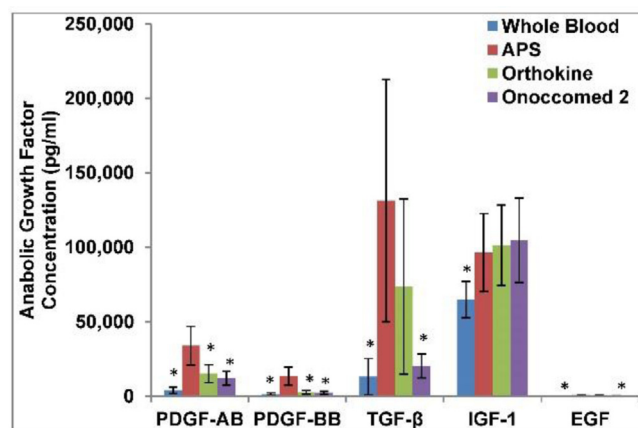


Figure 2. Anabolic growth factor concentrations.* significantly < APS Device System ($p < 0.05$, t-test).

test). There were no significant differences in the concentrations of EGF, insulin-like growth factor-1 (IGF-1), and TGF- β in the outputs of the APS and Orthokine device systems. However, the APS Device System produced solutions with significantly greater concentrations of PDGF-AB and PDGF-BB than the Orthokine Device System ($p < 0.05$, t-test).

Conclusions: The APS device system produced solutions with significantly greater concentrations of anti-inflammatory cytokines and similar or greater concentrations of anabolic growth factors than the Orthokine or Onocommed 2 Device Systems. Significantly, the average IL-1ra concentration in the output of the APS Device system was 34X that of the Orthokine Device System and 198X that of the Onocommed 2 Device System from the same donors. The cytokine profile of APS can be attributed to its increased concentrations of WBC and platelets compared to the acellular conditioned serums.

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DEVELOPMENT OF GROWTH FACTOR TETHERED HYALURONAN MICROSPHERES FOR IN SITU CHONDROGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

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Table 1

Concentration of white blood cells (WBC) (k/μl), Platelets (k/μl), and red blood cells (RBC) (M/μl)

	WBC (k/μl)	PLT (k/μl)	RBC (M/μl)
Whole blood	5.4 ± 1.8	175.1 ± 69.5	5.5 ± 1.1
APS	46.5 ± 19.9	1184.4 ± 556.4	1.4 ± 1.5
Orthokine	0.0 ± 0.0	13.5 ± 5.5	0.0 ± 0.0
Onocommed 2	0.0 ± 0.0	11.4 ± 1.7	0.0 ± 0.0

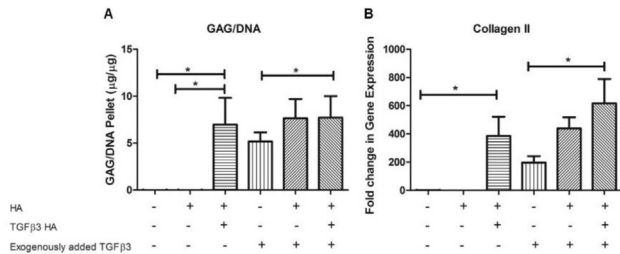


Figure 1: *In vitro* chondrogenesis of hMSCs. Results indicate a significant increase in GAG deposition and collagen type II expression with the addition of TGFβ3 HA microspheres. * $p \leq 0.05$

Purpose: Transforming growth factor beta (TGF-β) is an important regulator in determining cell fate of human mesenchymal stem cells (hMSCs), specifically down the chondrogenic lineage. Yet control of this differentiation within a complex *in vivo* milieu remains a challenge. A localized therapy such as an intra-articular injection of a system for controlled release of TGF-β3 would represent a readily translatable approach for *in situ* differentiation and remove the need for continuous supplementation of growth factors. Hyaluronan (HA) is an essential component of cartilage extracellular matrix (ECM) and serves as a support for cell adhesion, spreading and subsequently, cell growth. Thus, our aim was to fabricate chondromimetic HA microspheres and test these as extracellular growth factor targeting depots for initiation of *in vitro* and *ex vivo* chondrogenesis of hMSCs.

Methods: HA microspheres were fabricated using an electrostatic layer-by-layer strategy and characterized by scanning electron microscopy. hMSCs isolated from the iliac crest of healthy donors were obtained with approval from the National University of Ireland Galway and associated University College Hospital ethics committees and cultured in α -MEM containing 10% FBS, 100U/ml penicillin and 100µg/ml streptomycin. The effects of microspheres on cell number and metabolic activity were determined using the PicoGreen dsDNA® and alamarBlue™ assays. Confocal microscopy, flow cytometry and transmission electron microscopy were utilized to investigate co-localization of HA microspheres with hMSCs to ensure they were not taken up by cells. TGF-β3 was loaded onto HA microspheres via passive diffusion (400ng/mg), incubated with hMSCs and cultured for 21 d in incomplete chondrogenic medium (ICM) or complete chondrogenic medium (CCM) (+10ng/ml TGF-β3 every 2 d) to assess *in vitro* chondrogenesis. Glycosaminoglycan (GAG) measurement (DMMB assay), chondrogenic transcripts (qRT-PCR) and immunohistochemical staining (IHC) were used to validate successful chondrogenesis. For *ex vivo* culture experiments, fresh human articular cartilage samples were obtained under institutionally approved protocols from Merlin Park Hospital, Galway from donors who had undergone knee replacement surgery. Cartilage explants were allowed to equilibrate at 37°C for 48 h in Dulbecco's Modified Eagle's Medium (DMEM) + 10% fetal bovine serum and subsequently equilibrated for 24 h in ICM in an agarose well prior to switching to ICM or CCM media and treated with or without TGF-β3 loaded microspheres for 21 d culture. Experiments were performed for 3 donors with each experiment containing a minimum of three biological replicates. Statistical significance was assessed using one-way or

two-way ANOVA followed by Tukey or Bonferroni post hoc analysis and denoted significant for $p \leq 0.05$.

Results: HA microspheres retained their spherical morphology with uniform size distribution. Cellular compatibility studies indicated no adverse effects of 10µm HA microspheres. Following TGF-β3 loading of microspheres, incubation with hMSCs for 21 d *in vitro* pellet culture revealed enhanced accumulation of GAGs (Fig. 1A) and positive IHC of collagen type II in hMSCs incubated with growth factor loaded HA microspheres when compared to control cells. Additionally, qRT-PCR analysis from 14 d pellets revealed enhanced expression of aggrecan and collagen type II (Fig. 1B). 21 d explant culture indicated localization of TGF-β3 HA microspheres within fibrillations of osteoarthritic cartilage with implanted hMSCs and local chondrocytes showing increased GAG deposition compared to hMSCs alone (Fig. 2, arrow indicative of increased GAG staining in surrounding cells).

Conclusion: Injectable biomaterials hold great promise for orthopaedic applications, in particular for repair of articular cartilage. Preliminary *in vitro/ex-vivo* evidence presented here supports the use of TGF-β3-loaded HA microspheres for *in situ* differentiation of hMSCs.

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PRODUCING ARTIFICIAL CHONDRONS FOR IMPROVED CARTILAGE REPAIR

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Purpose: Articular cartilage has a limited capacity for self-repair and untreated damage often leads to the development of osteoarthritis and eventually joint failure. Currently, autologous chondrocyte implantation is the most successful method for repairing focal cartilage defects. This therapy involves the isolation of chondrocytes from articular cartilage by enzymatic degradation. This not only strips the cells from their extra cellular matrix, but also from their highly specialized micro-environments, called chondrons. This initiates dedifferentiation, i.e. the isolated chondrocytes progressively lose their chondrogenic phenotype, and thus reduces the clinical success of the therapy. We aim to develop a strategy to encapsulate expanded single chondrocytes in artificial chondrons. When successful, this approach represents a cost-effective method to prevent further dedifferentiation, while simultaneously allows for optimal stimulation of the encapsulated chondrocytes. Here, we present a microfluidic device able to encapsulate single chondrocytes in chondron-sized (<50 µm) microgels in a custom designable artificial matrix using an efficient and cell-friendly manner.

Methods: The master mould for the optimized microfluidic droplet generator was made by micropatterning ~25 µm thick SU8-50 (MicroChem) on a silicon wafer using standard photolithography techniques. PDMS (Sylgard 184, Dow Corning) was thermally cured on the master and bonded to glass after plasma treatment. Aquapel (Vulcavite) was introduced in the chip before usage to ensure hydrophobic channels walls. We compared various combinations of oils, surfactants, photoinitiators and UV dosages, as these are of paramount importance to obtain chondron-sized microgels. Chondron-sized droplets were produced by emulsifying a chondrocyte-laden hydrogel precursor solution of 10% (w/v) polyethylene glycol diacrylate (PEGDA, Laysan Bio, Inc.) and 0.1% (w/v) photoinitiator (Irgacure 2959, Ciba Specialty Chemicals) in hexadecane (Sigma) with 1% Span80 (Sigma). Subsequently, the microgels were formed in an on-chip delay channel by curing the emulsion with 365nm UV-light (Hamamatsu LC8).

Results: Chondrocytes were encapsulated in PEGDA microgels using droplet microfluidics at a rate of typically 20,000 cells per minute. The number of encapsulated cells followed a Poisson-distribution that was dependent on the cell concentration. We aimed to minimize the microgels' dimensions by comparing different combinations of oils, surfactants and UV dosages. Fluorinated oils in combination with PEGylated fluorosurfactants resulted in the most stable emulsions and thus allowed for the lowest photoinitiator concentration and UV dose for cell-laden microgel production. However, the use of fluorinated oils resulted in relatively large gels, which is a consequence of their low viscosity compared to the hydrogel precursor solution. In contrast, the use of a more viscous hydrocarbon oil with Span80 surfactant resulted in smaller, chondron-sized microgels. However, emulsions with this oil/surfactant combination were less stable off-chip. Solid on-chip cross-linking was accomplished by using an extended delay channel which enabled prolonged UV radiation without compromising the production rate. Finally, by varying the flow rates of the continuous and dispersed

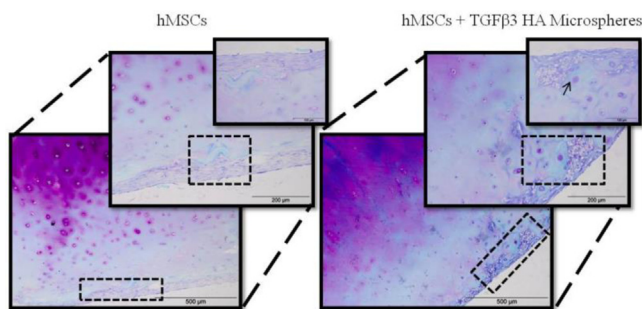


Figure 2: *Ex-vivo* culture of human articular cartilage explants for 21 d in ICM with human mesenchymal stems with or without TGFβ3 loaded HA microspheres.